

## REPORT DOCUMENTATION PAGE

Form Approved OMB NO. 0704-0188

The public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.  
PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.

1. REPORT DATE (DD-MM-YYYY) 20-12-2015	2. REPORT TYPE Final Report	3. DATES COVERED (From - To) 1-Sep-2014 - 31-May-2015		
4. TITLE AND SUBTITLE Final Report: Development of a Microfluidic Platform to Analyze Evolution of Programmed Bacterial Death		5a. CONTRACT NUMBER W911NF-14-1-0144		
		5b. GRANT NUMBER		
		5c. PROGRAM ELEMENT NUMBER 611102		
6. AUTHORS Lingchong You		5d. PROJECT NUMBER		
		5e. TASK NUMBER		
		5f. WORK UNIT NUMBER		
7. PERFORMING ORGANIZATION NAMES AND ADDRESSES Duke University 2200 West Main Street Suite 710 Durham, NC 27705 -4010		8. PERFORMING ORGANIZATION REPORT NUMBER		
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS (ES) U.S. Army Research Office P.O. Box 12211 Research Triangle Park, NC 27709-2211		10. SPONSOR/MONITOR'S ACRONYM(S) ARO		
		11. SPONSOR/MONITOR'S REPORT NUMBER(S) 65461-LS-II.2		
12. DISTRIBUTION AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited				
13. SUPPLEMENTARY NOTES The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision, unless so designated by other documentation.				
14. ABSTRACT  Evolution of programmed cell death in bacteria is a poorly understood phenomenon in biology. A critical limitation is the lack of high-throughput technology to examine dynamics of small bacterial populations. To address this limitation, we proposed to develop a droplet-based microfluidic technology to generate population ‘bottleneck’. <small>This platform will serve as a critical foundation for our long-term goal to develop a quantitative understanding of</small>				
15. SUBJECT TERMS Microfluidics, systems biology				
16. SECURITY CLASSIFICATION OF: a. REPORT UU		17. LIMITATION OF ABSTRACT UU	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON Lingchong You
b. ABSTRACT UU		c. THIS PAGE UU		19b. TELEPHONE NUMBER 919-660-8408

## Report Title

Final Report: Development of a Microfluidic Platform to Analyze Evolution of Programmed Bacterial Death

### **ABSTRACT**

Evolution of programmed cell death in bacteria is a poorly understood phenomenon in biology. A critical limitation is the lack of high-throughput technology to examine dynamics of small bacterial populations. To address this limitation, we proposed to develop a droplet-based microfluidic technology to generate population ‘bottleneck’. This platform will serve as a critical foundation for our long-term goal to develop a quantitative understanding of PAD evolution by using engineered bacteria. For the proposed pilot study, we will focus on the technology development. In particular, our proposed work consists of following specific tasks, focusing on proof-of-concept demonstration of this device and its use for analyzing engineered bacteria

---

**Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:**

**(a) Papers published in peer-reviewed journals (N/A for none)**

Received      Paper

11/03/2015 1.00 Jaydeep K. Srimani, Shuqiang Huang, Anna J. Lee, Ying Zhang, Allison J. Lopatkin, Kam W. Leong, Lingchong You. Dynamic control and quantification of bacterial population dynamics in droplets, *Biomaterials*, (08 2015): 0. doi: 10.1016/j.biomaterials.2015.05.038

**TOTAL:**      **1**

**Number of Papers published in peer-reviewed journals:**

---

**(b) Papers published in non-peer-reviewed journals (N/A for none)**

Received      Paper

**TOTAL:**

**Number of Papers published in non peer-reviewed journals:**

---

**(c) Presentations**

**Number of Presentations:**

---

**Non Peer-Reviewed Conference Proceeding publications (other than abstracts):**

Received      Paper

**TOTAL:**

**Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):**

---

**Peer-Reviewed Conference Proceeding publications (other than abstracts):**

Received      Paper

**TOTAL:**

**Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):**

---

**(d) Manuscripts**

Received      Paper

**TOTAL:**

**Number of Manuscripts:**

---

**Books**

Received      Book

**TOTAL:**

Received      Book Chapter

**TOTAL:**

---

**Patents Submitted**

---

**Patents Awarded**

---

**Awards**

---

**Graduate Students**

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
-------------	--------------------------

**FTE Equivalent:**

**Total Number:**

---

**Names of Post Doctorates**

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
-------------	--------------------------

Shuqiang Huang                    0.44

Kui Zhu                            0.33

**FTE Equivalent:**                    **0.77**

**Total Number:**                    **2**

### **Names of Faculty Supported**

<u>NAME</u>	<u>PERCENT_SUPPORTED</u>	National Academy Member
Lingchong You	0.04	
<b>FTE Equivalent:</b>	<b>0.04</b>	
<b>Total Number:</b>	<b>1</b>	

### **Names of Under Graduate students supported**

<u>NAME</u>	<u>PERCENT_SUPPORTED</u>
<b>FTE Equivalent:</b>	
<b>Total Number:</b>	

### **Student Metrics**

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: ..... 0.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:..... 0.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):..... 0.00

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense ..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:..... 0.00

### **Names of Personnel receiving masters degrees**

NAME

**Total Number:**

### **Names of personnel receiving PHDs**

NAME

**Total Number:**

### **Names of other research staff**

<u>NAME</u>	<u>PERCENT_SUPPORTED</u>
Su Ai Zhang	0.22
<b>FTE Equivalent:</b>	<b>0.22</b>
<b>Total Number:</b>	<b>1</b>

### **Sub Contractors (DD882)**

**Inventions (DD882)**

**Scientific Progress**

See attachment

**Technology Transfer**

Not applicable

W911NF-14-0144

## Development of a microfluidic platform to analyze evolution of programmed bacterial death

PI. Lingchong You, PhD  
Duke University

### Statement of the problem

Our long-term goal is to address the challenges mentioned above in two aspects. On one hand, we plan to use engineered gene circuits as well-defined model systems to develop a quantitative understanding of programmed altruistic death (PAD). On the other, we plan to develop a droplet-based microfluidic technology to generate population ‘bottleneck’. This platform will serve as a critical foundation for our long-term goal to develop a quantitative understanding of PAD evolution by using engineered bacteria. For the proposed pilot study, we focused on the technology development.

### Summary of the most important results

We have successfully accomplished the proposed objectives in the pilot project. The results of this research have been published in leading biomaterials journal, *Biomaterials*:

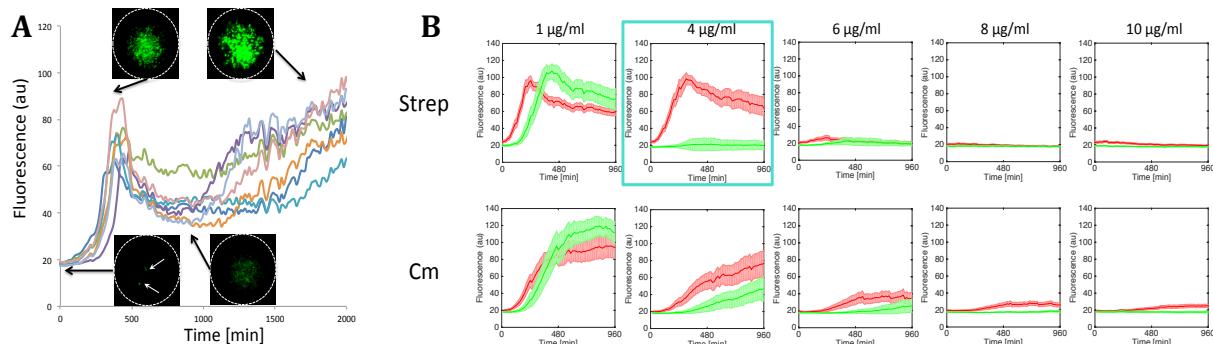
S. Huang, J. K. Srimani, A. J. Lee, Y. Zhang, A. J. Lopatkin, K. W. Leong and **L. You** (2015), Dynamic control and quantification of bacterial population dynamics in droplets. *Biomaterials*. 61:239-45. doi: 10.1016/j.biomaterials.2015.05.038.

Below, we summarize the key achievements described in detail in the paper.

### 1. Generating stable droplets to culture and quantify engineered bacteria

To validate the capability of our proposed droplet technology, we fabricated a microfluidic device to produce droplets, and used the droplets to quantify two different population dynamics.

**Programmed population control by an ePop circuit:** We first use the droplet platform to quantify the population dynamics of an engineered *E. coli* MC4100Z1 carrying the ePop circuit that we previously engineered. The ePop cells generated oscillatory dynamics in droplets similar to previous observation in bulk cultures (**Figure 1A**)

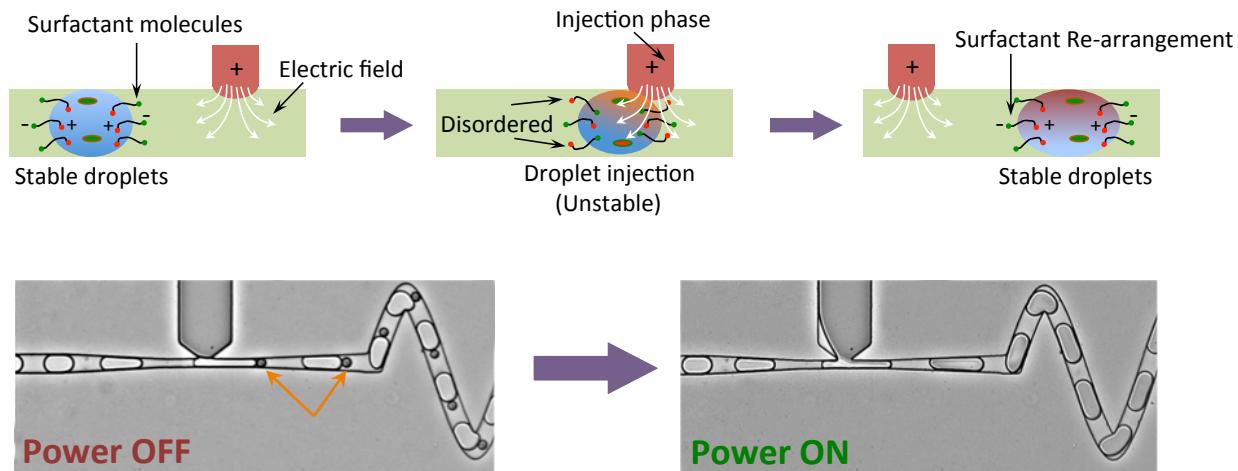


**Figure 1. (A) Quantification of multiple droplets with oscillating behaviors.** Each line represents one subpopulation in droplet started with low cell density (1~3 cells per droplet). Images are the representative time points of ePop oscillation. **(B) Investigation of IE in droplets.** The engineered bacteria with GFP reporter were encapsulated in droplets with varied concentrations of antibiotics. The curves indicate mean fluorescence intensity of sampled droplets ( $n > 20$ ) versus time, and shades refer to the standard deviation.

**Inoculum effect in response to antibiotics:** We also investigated the inoculum effect (IE) of a lab bacterial strain within droplets. The sensitive bacterial strain BW25113 with a GFP reporter was encapsulated in the droplets at a high and low initial cell densities, respectively. When the antibiotic concentration was below 4 $\mu$ g/ml, droplets with both high and low initial cell densities grew to a high final density. When the antibiotic concentration was above 4 $\mu$ g/ml, neither of them could grow (**Figure 1B** top row). Importantly, when the antibiotic concentration was 4 $\mu$ g/ml, the droplets with a high initial density survived, while those with a low initial density did not. This density-dependent survival is the defining character of IE. In contrast, the antibiotics that do not induce HSR and rapid ribosome degradation will not cause IE (**Figure 1B** bottom row). Droplets with both high and low cell densities either grew ( $[C_m] \leq 4\mu\text{g}/\text{ml}$ ) or were inhibited ( $[C_m] > 4\mu\text{g}/\text{ml}$ ).

## 2. Demonstrating a droplet injection method to control the droplet environment.

As the original proposal, we introduced a droplet merging method to control the growth dynamics within droplets. Herein, we used an electrode-free injection technology to manipulate the droplet environment that was controlled by a DC power source. When the power source is OFF, the generated droplet is stabilized by surfactant molecules and the injection does not occur. However, when the power source is ON, the electricity field destabilizes the surfactant layer of each droplet as it passes the orifice. The injection channel can then deliver a small amount of liquid into the droplet, forming a larger one (**Figure 2**).

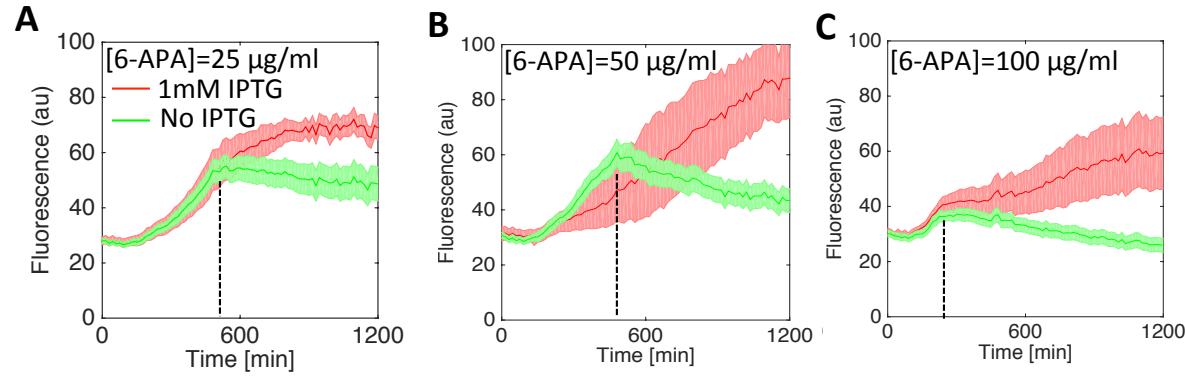


**Figure 2. Mechanism of droplet injection.** A layer of surfactant molecules stabilizes the droplets after production. An electric field disrupts this protecting layer when power is turned ON, and the injection phase is injected into the original droplets when they move across the orifice. Real-time images depict the difference between power OFF and ON. Orange arrows indicate the individual droplets from injection phase without being injected.

## 3. Applying optimized droplet injection method to precise control growth dynamics in droplets.

We next tested the PAD dynamics in the droplets. We modulated the final concentrations of 6-APA and IPTG in droplets by varying their concentrations in the injection phase. At 25 $\mu$ g/ml 6-APA, the population without any induction of BlaM production initiated growth for the first 510min but stopped growing thereafter (green line in **Figure 4A**); in comparison, when the population was induced by 1mM IPTG, the cells grew to a higher density; this is due to the degradation of 6-APA by BlaM (red line in **Figure 4A**). Similarly, when the concentration of 6-APA was increased to

50 $\mu$ g/ml, the discrepancy between un-induced (green line in **Figure 4B**) and induced populations (red line in **Figure 4B**) was more significant. For the population without IPTG induction, the inflection point of population growth was slightly shifted to shorter time due to higher stress compared to 25 $\mu$ g/ml 6-APA. However, if the concentration of 6-APA was further increased to 100 $\mu$ g/ml, the discrepancy between the two populations appeared to decrease (**Figure 4C**). This could be explained by the fact that the stress was too high, and the released BlaM could not efficiently remove the antibiotic. The stress level was also reflected by the inflection point of population growth without IPTG, which shifted to 250min (green line in **Figure 4C**).



**Figure 3.** (A) 25 $\mu$ g/ml (B) 50  $\mu$ g/ml and (C) 100 $\mu$ g/ml 6-APA was injected into droplets with (red) and without (green) 1mM IPTG rescuing. Black dash line indicates inflection point when the population stops growing without IPTG.